Nitric Oxide Donors Increase Blood Flow and Reduce Brain Damage in Focal Ischemia: Evidence that Nitric Oxide is Beneficial in the Early Stages of Cerebral Ischemia

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Summary: We studied whether administration of nitric oxide (NO) donors reduces the ischemic damage resulting from middle cerebral artery (MCA) occlusion in spontaneously hypertensive rats (SHRs). In halothane-anesthetized and ventilated SHRs, the MCA was occluded. CBF was monitored using a laser–Doppler flowmeter. Three to five minutes after MCA occlusion, the NO donors sodium nitroprusside (SNP; 3 mg/kg/h) or 3-morpholino-sydnonimine (SIN 1; 1.5–6 mg/kg/h) were administered into the carotid artery for 60 min. As a control, the effect of papaverine (3.6 mg/kg/h), a vasodilator that acts independently of NO, was also studied. The hypotension evoked by these agents was counteracted by intravenous infusion of phenylephrine. At the end of the infusion, rats were allowed to recover. Stroke size was determined 24 h later in thionin-stained sections. In sham occluded rats, SNP (n = 5), SIN 1 (n = 5), and papaverine (n = 5) produced comparable increases in CBF (p > 0.05 from vehicle). After MCA occlusion, SNP (n = 5) and SIN 1 (n = 5), but not papaverine (n = 5), enhanced the recovery of CBF (p < 0.05 from vehicle) and reduced the size of the infarct by 28 ± 12 and 32 ± 7%, respectively (mean ± SD; p < 0.05 from vehicle). To determine whether NO donors could act by inhibiting platelet aggregation, we studied the effect of SNP on collagen-induced platelet aggregation. Intracarotid administration of SNP (3 mg/kg/h for 60 min) did not affect platelet aggregation to collagen, suggesting that the protective effect of NO donors was not due to inhibition of platelet function. We conclude that NO donors increase CBF to the ischemic territory and reduce the tissue damage resulting from focal ischemia. The protective effect may result from an increase in CBF to the ischemic territory, probably the ischemic penumbra. These findings suggest that NO donors may represent a new therapeutic strategy for the management of acute stroke. **Key Words:** Cerebral blood flow—Laser–Doppler flowmetry—Middle cerebral artery—3-Morpholino-sydnonimine—Platelet aggregation—Sodium nitroprusside.

Nitric oxide (NO) is a ubiquitous free radical gas involved in a wide variety of biological processes (Moncada, 1992). In the systemic circulation, NO, produced in endothelial cells, regulates vascular resistance and platelet aggregation (Moncada, 1992). In the immune system, NO is responsible for the cytotoxicity of activated macrophages (Nathan and Hibbs, 1991). In the nervous system, neuronal NO may act as a neurotransmitter (Dawson et al., 1992) and may participate in the regulation of the cerebral circulation (Iadecola, 1993). NO is synthesized from L-arginine by the enzyme NO synthase, an enzyme of which three isoforms have been thus far identified (Moncada, 1992).

Recent studies have suggested that NO may play a role in the expression of cerebral ischemic damage. Whether NO is beneficial or detrimental to the ischemic tissue, however, has not been clearly established. NO, a potent cerebrovasodilator and inhibitor of platelet aggregation (for review see Radomski et al., 1991; Iadecola, 1993), may increase

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CBF in the ischemic territory and ameliorate isch-emic damage (Morikawa et al., 1992*a,b;* Zhang and Iadecola, 1993*a*). On the other hand, it is well es-tablished that NO is cytotoxic (Nathan and Hibbs, 1991; Dawson et al., 1992), and some studies have presented evidence that NO is a key factor in *N*-methyl-D-aspartate (NMDA)–mediated brain injury and in ischemic neuronal death (Dawson et al., 1991; Nowicki et al., 1991; Buisson et al., 1992; Nagafuji et al., 1992). Therefore, the precise role of NO in cerebral ischemia remains to be established.

To investigate further the role of NO in the mech-anisms of cerebral ischemic damage, we studied the effect of NO donors, agents that generate NO (Feelisch, 1991), on the magnitude of the brain dam-age resulting from occlusion of the middle cerebral artery (MCA) in rat. We found that administration of NO donors improves CBF in the ischemia area and reduces the size of the infarct. NO donors did not affect platelet aggregation to collagen. These findings indicate that NO is beneficial in the acute phases of focal cerebral ischemia and raise the pos-sibility that NO donors could be important in the management of acute ischemic stroke.

METHODS

Methods for MCA occlusion, recording and analysis of the EEG, monitoring of CBF by laser–Doppler flowmetry (LDF), and determination of infarct size have been de-scribed in detail in previous publications (Zhang and Ia-decola, 1992, 1993*a,b*) and will only be summarized here.

General surgical procedures

Studies were conducted on 57 male spontaneously hy-pertensive rats (SHRs) (Harland) and 17 Sprague–Dawley rats weighing 300–400 g. Animals were not fasted. Anes-thesia was induced with halothane (5% in 100% oxygen) administered through a facial mask. When deeply anes-thetized, rats were intubated transorally as previously de-scribed (Zhang and Iadecola, 1993*b*). Briefly, the epiglot-tis and vocal cords were visualized with the aid of a fi-beroptic illuminator, and a polyethylene catheter (OD 2.8 mm) was gently advanced through the oral cavity into the trachea. The left external carotid artery was cannulated centripetally and the catheter advanced toward the ca-rotid bifurcation and secured in place (Fig. 1). The fem-oral vessels were also cannulated (Zhang and Iadecola, 1993*a*). Rats were placed on a stereotaxic frame (Kopf) and artificially ventilated with 100% oxygen by a mechan-ical ventilator (Harvard Apparatus Rodent Respirator). At this time, the halothane concentration was reduced to 1.0%. Ventilation with 100% O_2 was used because in pre-liminary studies it was found that hyperoxia facilitates the recovery of the animals after extubation. The body tem-perature was maintained at 37 $\pm$ 0.5°C by a thermostati-cally controlled infrared lamp connected to a rectal probe. One of the femoral arterial catheters was used for continuous recording of arterial pressure (AP), mean AP, and heart rate. The other catheter was used for blood
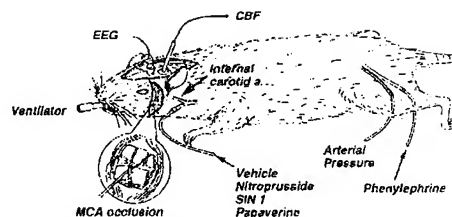
FIG. 1. Drawing illustrating the experimental protocol used in this study. See Methods for details. MCA, middle cerebral artery; SIN 1, 3-morpholino-sydnonimine.

sampling for measurement of P_aO_2, P_aCO_2, and pH by a blood gas analyzer (Corning model 178).

Monitoring of CBF and EEG

CBF was monitored in the cerebral cortex ipsilateral to the occluded MCA using LDF (Vasamedic, BPM 403A) (Zhang and Iadecola, 1993*a,b*). A small hole 1–2 mm in diameter was drilled with the assistance of a dissecting scope at a site 2.5–3 mm lateral to the midline and 4.2 $\pm$ 0.7 mm rostral to the interaural line. This site was found in preliminary studies to correspond to the region of the cerebral cortex that is rescued from infarction (see fol-lowing). The dura was left intact and the LDF probe (tip diameter 0.8 mm) was positioned 0.5 mm above the dural surface. The analog output of the flowmeter was fed into a DC amplifier (Grass) and displayed on the polygraph. The EEG was recorded monopolarly from a stainless-steel screw inserted through to the skull at a site 2 mm lateral to midline and 1 mm rostral to the LDF probe. The reference electrode was a clip attached to the scalp.

Occlusion of MCA and determination of infarct volumes

Procedures for proximal or distal MCA occlusion and for determination of the infarct volume are identical to those published previously (Zhang and Iadecola, 1992, 1993*a,b*). Briefly, for MCA occlusion a 3- to 4-mm hole was drilled at a site superior and lateral to the foramen ovale to expose the proximal or distal portion of the MCA. The MCA was elevated and cauterized either prox-imal to the origin of the lenticulostriate branches or me-dial to the inferior border of the inferior cerebral vein (Fig. 1) (Zhang and Iadecola, 1992). Twenty-four hours after MCA occlusion, rats were killed and their brains were removed. The site of CBF recording was marked and the brain was frozen in isopentane. Coronal sections (thickness 30 μm) were serially cut in a cryostat, col-lected at 300-μm intervals, and stained with thionin. In-farct volume was determined using an image analyzer (MCID; Imaging Research) as previously described (Zhang and Iadecola, 1992, 1993*a,b*).

Platelet aggregation studies

Platelet aggregation was assessed ex vivo as previously described (Rao et al., 1983). Briefly, platelet-rich plasma was obtained by centrifugation (200 *g* for 20 min at room temperature) of heparinized (12.5 U/ml) arterial blood. Platelet aggregation was tested with a Chronolog Lumiag-gregometer using collagen (10–20 μg/ml) as an aggregating agent.

Experimental protocol

Effect of NO donors on focal ischemic damage. SHRs were surgically prepared and the MCA was exposed as described. Arterial blood gases were adjusted so that arterial Pco_2 was between 33 and 38 mm Hg (Tables 1 and 2). The stereotaxic frame was rotated to place the animal in a lateral position and the MCA occluded as described. Occurrence of neocortical ischemia was indicated by a sudden drop in CBF associated with a more gradual reduction in the amplitude of the EEG. Three to five minutes after MCA occlusion, normal saline (vehicle), sodium nitroprusside (SNP; Sigma; 3 mg/kg/h), 3-morpholino-sydnonimine (SIN 1; Cassella, AG; 3 mg/kg/h) or papaverine (Sigma; 3.6 mg/kg/h) was infused into the external carotid catheter for 1 h at a rate of 1 ml/h. All drugs were dissolved in saline and solutions were used fresh. SNP and SIN 1 generate NO and are potent vasodilators (Feelisch, 1991). The concentrations of SNP, SIN 1, and papaverine were selected in preliminary studies so as to elicit comparable increases in CBF when administered into the carotid artery (Fig. 2). To counteract the hypotension associated with infusion of NO donors or papaverine, an intravenous infusion (0.5–1 ml/h) of phenylephrine (10–100 μg/h) was also begun. In rats receiving intracarotid infusion of vehicle, saline was administered intravenously. Therefore, AP did not differ among animals receiving vehicle, NO donors, or papaverine (Tables 1 and 2). At the end of the infusion period, animals were monitored to ensure that the AP was stable. Catheters were then removed and wounds infiltrated with lidocaine and sutured. Animals were allowed to recover and carefully observed during the postoperative period for the occurrence of seizures. Twenty-four hours later, rats were killed for determination of infarct size.

Several groups of SHRs with or without MCA occlusion were studied (Tables 1 and 2). The first four groups consisted of rats in which the MCA was exposed but not occluded (sham occlusion). These animals received intracarotid infusion of vehicle (n = 5), SNP (3 mg/kg/h; n = 5), SIN 1 (3 mg/kg/h; n = 5), or papaverine (3.6 mg/kg/h; n = 5). In six groups of rats, the MCA was occluded distally. These animals were treated with vehicle (n = 6), SNP (3 mg/kg/h; n = 5), SIN 1 (1.5 mg/kg/h; n = 4), SIN 1 (3 mg/kg/h; n = 5), SIN 1 (6 mg/kg/h; n = 4), or papaverine (3.6 mg/kg/h; n = 5). In two additional groups, the MCA was occluded proximal to the origin of the len-

ticulostriate branches. These animals received vehicle (n = 4) or SNP (3 mg/kg/h; n = 4).

Effect of nitroprusside on platelet aggregation. In these experiments, the effect of intracarotid infusion of SNP (3 mg/kg/h) on platelet aggregation was studied. Sprague–Dawley rats (n = 5) were anesthetized with halothane and surgically prepared for infusion of SNP into the left carotid artery. Prior to the infusion of SNP, a sample of arterial blood was collected (3 ml). The infusion of SNP (3 mg/kg/h) was then started. Phenylephrine was coinfused intravenously to counteract the hypotension elicited by SNP. One hour later, a second arterial blood sample was collected and the SNP infusion was stopped. Blood samples were then processed for platelet aggregation as described. In other experiments SNP (500–1,000 μM) was added directly to platelet-rich plasma from untreated donor rats (n = 3) and platelet aggregation was then tested.

Data analysis

Data are expressed as means ± SD. Comparisons among multiple groups were statistically evaluated by analysis of variance and Tukey's test (Systat, Inc.). Differences were considered significant for $p < 0.05$.

RESULTS

Effects of NO donors on CBF in sham-occluded rats

In these experiments the effects of intracarotid infusion of vehicle, SNP, SIN 1, and papaverine were established in animals in which the MCA was exposed but not occluded (sham occlusion). In rats receiving vehicle (n = 5), CBF was stable throughout the period of infusion (Fig. 2). Intracarotid infusion of SNP (n = 5), SIN 1 (n = 5), or papaverine (n = 5) produced comparable increases in CBF that were sustained throughout the period of infusion (+192 ± 64% for SNP at 60 min; $p < 0.05$, analysis of variance). In these sham-occluded groups, a small area of necrosis in the cortex adjacent to the surgical site was observed 24 h later (Table 3).

TABLE 1. *Arterial pressure, blood gases, and hematocrit of the groups of spontaneously hypertensive rats studied*

| | Min | Sham-Occlusion | | | |
		Vehicle	SNP (3 mg/kg)	SIN 1 (3 mg/kg)	Papaverine (3.6 mg/kg)
Arterial pressure (mm Hg)	0	133 ± 18	130 ± 8	145 ± 13	131 ± 17
	10	133 ± 24	133 ± 8	137 ± 14	135 ± 17
	30	130 ± 20	126 ± 7	128 ± 14	138 ± 13
	60	132 ± 18	122 ± 12	129 ± 8	133 ± 12
Pco_2 (mm Hg)		35.4 ± 1.6	35.8 ± 1.9	34.2 ± 1.8	35.1 ± 3
Po_2 (mm Hg)		501 ± 53	518 ± 12	427 ± 71	511 ± 6
pH		7.49 ± 0.05	7.45 ± 0.04	7.49 ± 0.04	7.48 ± 0.04
Hematocrit (%)	0	48 ± 3	48 ± 2	50 ± 1	48 ± 1
	60	49 ± 2	48 ± 1	50 ± 2	49 ± 2
n		5	5	5	5

Data are means ± SD; $p > 0.05$ (analysis of variance and Tukey's test). Arterial pressure values refer to the time before (0) and 10, 30, and 60 min after the start of the infusion of vehicle or drugs. Hematocrit was measured before (0) and at the end of the infusion (60) ($p > 0.05$, paired t-test). Abbreviations: MCA, middle cerebral artery; SNP, sodium nitroprusside; SIN 1, 3-morpholino-sydnonimine.

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TABLE 2. *Arterial pressure, blood gases, and hematocrit of the groups of spontaneously hypertensive rats studied*

			MCA occlusion (distal)						MCA occlusion (proximal)	
	Min	Vehicle	SNP (3 mg/kg)	SIN 1 (1.5 mg/kg)	SIN 1 (3 mg/kg)	SIN 1 (6 mg/kg)	Papaverine (3.6 mg/kg)	Min	Vehicle	SNP (3 mg/kg)
Arterial	0	123 ± 9	125 ± 10	138 ± 5	134 ± 14	129 ± 17	132 ± 8	0	121 ± 5	124 ± 11
pressure	10	127 ± 7	124 ± 4	148 ± 3	128 ± 13	136 ± 14	130 ± 14	10	129 ± 15	123 ± 13
(mm Hg)	30	130 ± 9	116 ± 8	135 ± 3	136 ± 12	129 ± 5	140 ± 10	30	125 ± 14	114 ± 5
	60	130 ± 8	111 ± 8	134 ± 9	123 ± 13	128 ± 10	133 ± 13	60	126 ± 9	113 ± 6
Pco_2 (mm Hg)		33.4 ± 3	34.6 ± 3	36.1 ± 2	35.7 ± 3	37.3 ± 1.3	35.9 ± 3		36.2 ± 1.5	33.5 ± 2.3
Po_2 (mm Hg)		465 ± 48	489 ± 40	510 ± 12	447 ± 49	426 ± 87	423 ± 82		483 ± 31	490 ± 29
pH		7.44 ± 0.04	7.44 ± 0.08	7.49 ± 0.05	7.48 ± 0.11	7.45 ± 0.05	7.47 ± 0.03		7.45 ± 0.03	7.47 ± 0.02
Hematocrit	0	47 ± 3	47 ± 2	51 ± 2	49 ± 2	49 ± 1	47 ± 1	0	48 ± 3	47 ± 4
(%)	60	47 ± 2	48 ± 3	49 ± 1	50 ± 2	49 ± 2	46 ± 2	60	47 ± 4	47 ± 2
n		6	5	4	5	4	5		4	4

Data are means ± SD; $p > 0.05$ (analysis of variance and Tukey's test). Arterial pressure values refer to the time before (0) and 10, 30, and 60 min after the start of the infusion of vehicle or drugs. Hematocrit was measured before (0) and at the end of the infusion (60) ($p > 0.05$, paired t-test). Abbreviations: MCA, middle cerebral artery; SNP, sodium nitroprusside; SIN 1, 3-morpholino-sydnonimine.

Effect of NO donors on recovery of CBF and on infarct resulting from distal MCA occlusion

In rats receiving vehicle (n = 6), distal occlusion of the MCA resulted in an immediate drop in CBF ($-77 \pm 11\%$). Sixty minutes later, CBF recovered by $10 \pm 3\%$ (Fig. 3). Twenty-four hours later, these animals had large infarcts involving predominantly the neocortex (Table 3; Fig. 4). The distribution of the infarct was similar to that previously reported in SHRs (Coyle, 1986).

In rats in which the effect of SNP or SIN 1 was studied, distal MCA occlusion resulted in an initial reduction in CBF similar to that of the animals that received vehicle ($p > 0.1$, analysis of variance). However, infusion of SNP (3 mg/kg/h; n = 5) or SIN 1 (3 mg/kg/h; n = 5) enhanced the recovery of CBF substantially (Fig. 3; $p < 0.05$ from vehicle at

FIG. 2. Effect of intracarotid administration of vehicle [saline; 1 ml/h (O)], sodium nitroprusside [3 mg/kg/h (□)], 3-morpholino-sydnonimine [SIN 1; 3 mg/ml/h (▲)], or papaverine [3.6 mg/ml/h (■)] on CBF in spontaneously hypertensive rats in which the middle cerebral artery was exposed but not occluded. See Fig. 1 for details on the experimental protocol. Nitroprusside, SIN 1, or papaverine infusion resulted in comparable increases in CBF that were sustained throughout the period of infusion (*$p < 0.05$ from vehicle, analysis of variance and Tukey's test).

20, 40, and 60 min). These animals had a significant reduction in stroke size at 24 h. The reduction averaged $28 \pm 12\%$ for SNP and $32 \pm 7\%$ for SIN 1 (3 mg/kg/h) ($p < 0.05$ from vehicle; Table 3). To determine whether the reduction in stroke size was related to the dose of SIN 1, two additional concentrations were tested. The effect of SIN 1 was apparent at a dose of 1.5 mg/kg/h (infarct reduction $-11 \pm 8\%$; n = 4; $p < 0.05$). At 3 mg/kg/h the reduction ($-32 \pm 7\%$) was not different from that seen at 6 mg/kg/h ($-32 \pm 11\%$; $p > 0.05$, n = 5). In contrast to SNP and SIN 1, infusion of papaverine (3.6 mg/kg/h) did not affect CBF recovery or stroke size ($p > 0.05$ from vehicle; Table 3, Figs. 3–5).

The distribution of the cross-sectional area of the infarct in rats receiving vehicle, SNP, SIN 1, or papaverine is illustrated in Figs. 4 and 5. The tissue retrieved from infarction by SIN 1 (3 mg/kg/h) was located at all rostrocaudal levels (Figs. 4 and 5). A similar distribution was observed in rats in which SNP was administered ($p > 0.05$ from SIN 1) (Fig. 4). In contrast to SIN 1 and SNP, papaverine did not affect the rostrocaudal distribution of the cross-sectional area of the infarct ($p > 0.05$ from vehicle) (Figs. 4 and 5).

To rule out the possibility that the reduction in infarct size was a consequence of effects on plasma glucose (Prado et al., 1988), we studied, in separate SHRs (n = 5), whether infusion of SIN 1 (3 mg/kg/h) modified plasma glucose. After MCA occlusion, plasma glucose was 217 ± 29 mg/dl before SIN 1 infusion and 267 ± 33 mg/dl at the end of the infusion (n = 5, $p < 0.05$). The increase in plasma glucose during SIN 1 administration is likely to be a consequence of the phenylephrine infusion given to prevent hypotension. These plasma glucose concentrations are higher than those previously re-
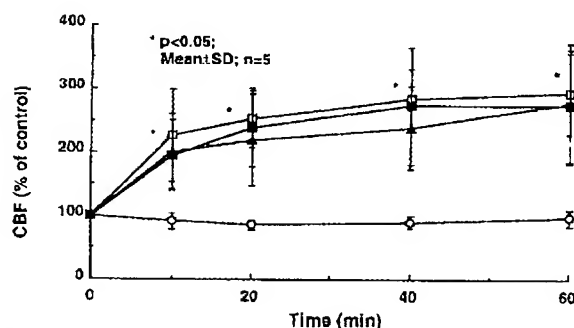
TABLE 3. *Effect of nitric oxide donors or papaverine on the volume of the infarct (mm^3) resulting from distal or proximal MCA occlusion in spontaneously hypertensive rats*

| | Sham occlusion | | | | MCA occlusion | | | | | |
| | | | | | Distal | | | | Proximal | |
	Vehicle	SNP (3 mg/kg)	SIN 1 (3 mg/kg)	Papaverine (3.6 mg/kg)	Vehicle	SNP (3 mg/kg)	SIN 1 (3 mg/kg)	Papaverine (3.6 mg/kg)	Vehicle	SNP (3 mg/kg)
Total	12 ± 9	18 ± 3	13 ± 4	15 ± 4	223 ± 18	161 ± 28[a]	152 ± 15[a]	230 ± 10	353 ± 16	292 ± 12[a]
Neocortex	12 ± 9	18 ± 3	13 ± 4	15 ± 4	212 ± 17	155 ± 27[a]	145 ± 14[a]	225 ± 9	269 ± 18	217 ± 11[a]
Striatum	0	0	0	0	6 ± 4	3 ± 3	4 ± 3	4 ± 5	65 ± 3	61 ± 6
n	5	5	5	5	6	5	5	5	4	4

Data are means ± SD.

[a] $p < 0.05$ from corresponding region in vehicle and papaverine group (analysis of variance and Tukey's test).

ported in SHRs (e.g., Morikawa et al., 1992a). Differences in the anesthesia protocol could perhaps account for the discrepancy. In the study of Morikawa et al. (1992a), anesthesia was maintained with halothane and 70% nitrous oxide, whereas in the present study only halothane was used.

To examine the possibility that the decrease in infarct size produced by NO donors was a consequence of a reduction in brain temperature (Morikawa et al., 1992c), the effect of intracarotid infusion of SIN 1 on brain temperature was studied in four intact Sprague-Dawley rats. Brain temperature was measured using a thermal probe (diameter 0.6 mm) inserted into the body of the caudate nucleus ipsilateral to the side of the infusion. Infusion of SIN 1 (3 mg/kg/h) increased cortical CBF but did not affect the temperature of the caudate nucleus (before infusion: 36.8 ± 0.7°C; during infusion: 36.8 ± 0.6°C). Therefore, infusion of SIN 1 did not result in brain hypothermia.
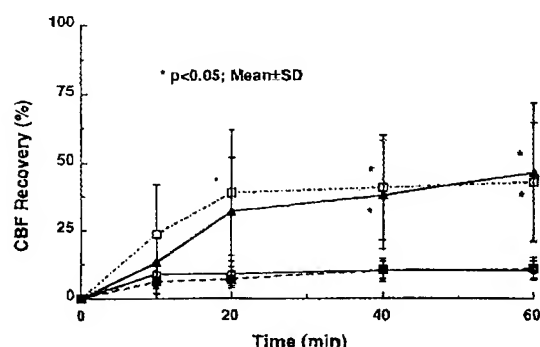

FIG. 3. Effect of intracarotid administration of vehicle [saline; 1 ml/h, n = 6 (-O-)], sodium nitroprusside (3 mg/kg/h, n = 5 (---□---)], 3-morpholino-sydnonimine [SIN 1; 3 mg/ml/h, n = 5 (-▲-)], or papaverine [3.6 mg/ml/h, n = 5 (--■--)] on CBF in spontaneously hypertensive rats in which the middle cerebral artery (MCA) was occluded. CBF recovery is presented as percentage of the initial reduction in CBF resulting from MCA occlusion. The infusion of drugs was started 3–5 min after MCA occlusion. Administration of nitroprusside or SIN 1 resulted in a significant improvement in the recovery of CBF (*p < 0.05 from vehicle, analysis of variance and Tukey's test). In contrast, infusion of papaverine did not affect CBF recovery (p > 0.05 from vehicle).

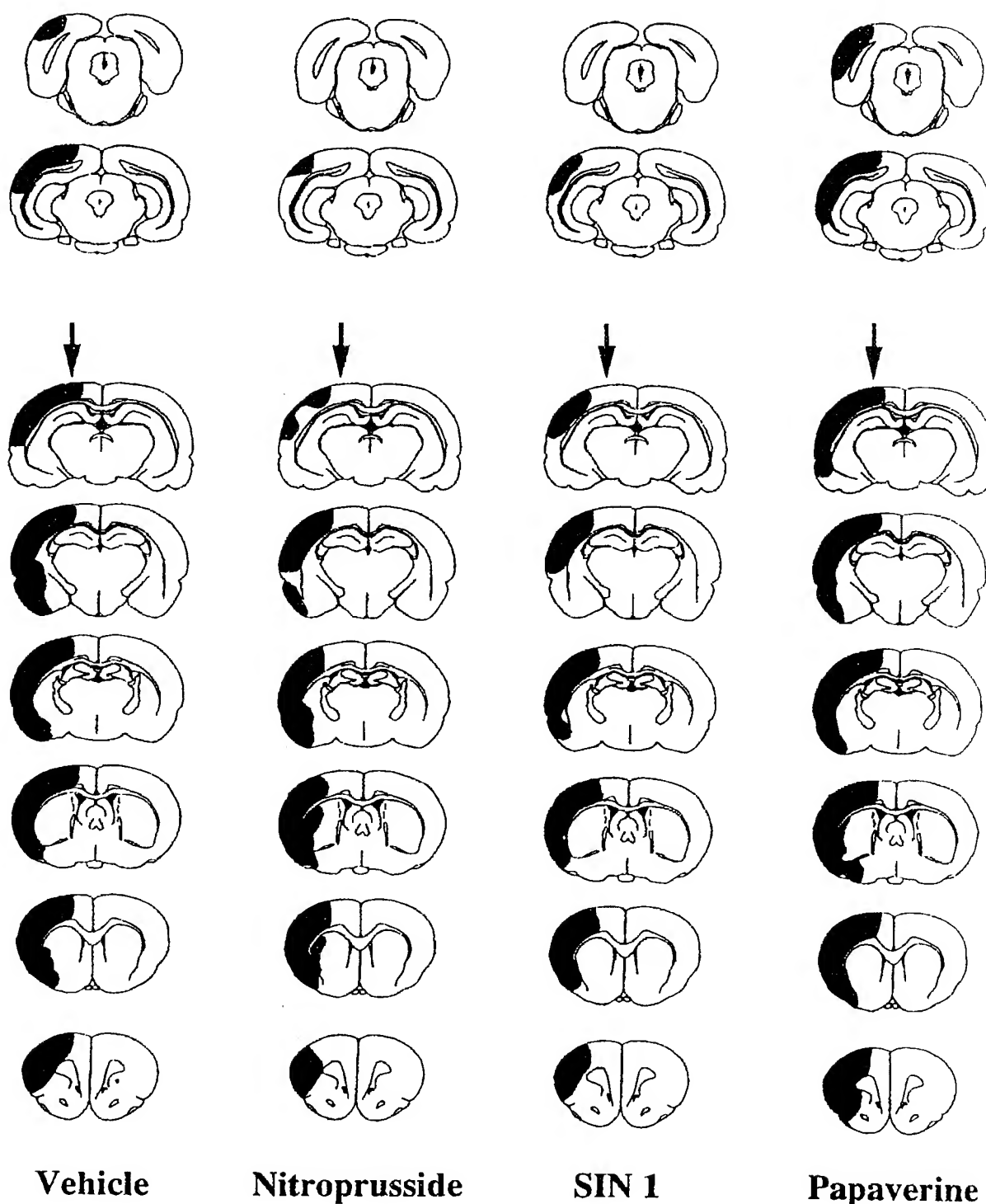### Relationship between location of CBF probe and tissue retrieved from infarction

The position of the laser–Doppler probe was histologically verified in all rats so that the outcome of the tissue in which CBF was recorded could be determined. The distribution of the infarcted tissue in reference to the location of the CBF probe in representative cases is illustrated in Fig. 4. In rats receiving vehicle or papaverine, the probe was positioned over tissue that underwent infarction (Fig. 4). In contrast, in animals that received SNP or SIN 1, the tissue located under the probe did not undergo infarction (Fig. 4). Therefore, the pattern of CBF recovery observed with SNP and SIN 1 reflects that of the ischemic region that was rescued from infarction.

Effect of SNP on ischemic damage after proximal occlusion of MCA

To determine whether the reduction in stroke size afforded by NO donors was restricted to the cerebral cortex or could involve the striatum as well, the effect of SNP on the stroke resulting from proximal MCA occlusion was studied. Occlusion of the MCA proximal to the lenticulostriate branches resulted in infarction involving both neocortex and striatum (Table 3). Administration of SNP (3 mg/kg/h) reduced stroke size by 19 ± 4% in the cortex (p < 0.05 from vehicle; Table 3), but did not affect stroke size in striatum (p > 0.05).

Effect of SNP on platelet aggregation

NO is a potent inhibitor of platelet aggregation (Radomski et al., 1991). To determine whether the reduction in stroke size exerted by NO donors was due to the antiplatelet effect of NO, we studied the effect of SNP on platelet aggregation ex vivo. Infusion of SNP (3 mg/kg/h) for 60 min in five rats did not influence platelet aggregation (Fig. 6). In contrast, SNP (500–1,000 μM) added directly to platelet-rich plasma inhibited aggregation induced by collagen (10–20 μg/ml) (Fig. 6). Therefore, infusions of SNP at a concentration effective in reducing

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Vehicle   Nitroprusside   SIN 1   Papaverine

FIG. 4. Distribution of the cross-sectional area of the infarct resulting from distal occlusion of the middle cerebral artery (MCA) in animals receiving vehicle, sodium nitroprusside, 3-morpholino-sydnonimine (SIN 1), or papaverine. Infusion of nitroprusside or SIN 1, but not papaverine, leads to smaller infarcts. The area rescued from infarction involves the dorsomedial aspect of the frontal, parietal, and occipital cortex. The arrows indicate the position of the laser–Doppler probe used to monitor CBF after MCA occlusion. In rats receiving vehicle or papaverine, the CBF probe overlies infarcted tissue, while in rats receiving nitroprusside or SIN 1, the probe is located over tissue that did not undergo infarction. As illustrated in Fig. 3, infusion of nitroprusside or SIN 1 enhanced the recovery of CBF in the tissue that was spared from infarction, while papaverine did not. The finding suggests that nitric oxide donors increase CBF to the tissue that is rescued from infarction.
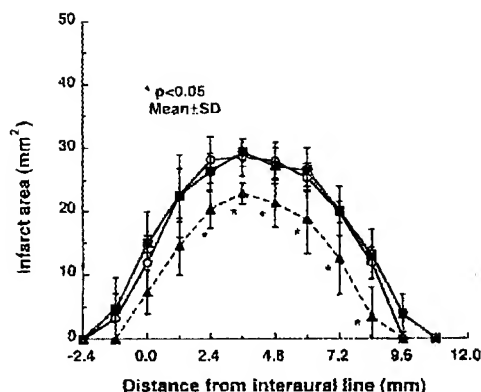
FIG. 5. Cross-sectional area of the infarct resulting from middle cerebral artery occlusion plotted as a function of the distance from the interaural line. Note that 3-morpholinosydnonimine [SIN 1, n = 5 (--▲--)], but not papaverine [n = 5 (-■-)] reduces the cross-sectional area of the infarct at most rostrocaudal levels. -O-, vehicle (n =6). *p < 0.05 (analysis of variance and Tukey's test).

stroke size do not influence collagen-induced platelet aggregation.

DISCUSSION

We have demonstrated that intracarotid administration of SNP and SIN 1, potent vasodilators that act by generating NO (Feelisch, 1991), reduces the brain damage resulting from MCA occlusion in SHRs. Administration of these agents increases CBF in the tissue that is rescued from infarction. In contrast, papaverine, a cerebrovasodilator that acts independently of NO (Iadecola, 1992a), fails to enhance CBF recovery or reduce infarct size. These findings indicate that NO donors ameliorate the functional impairment and tissue damage resulting from focal cerebral ischemia.

We have recently reported that administration of SNP reduces the infarct resulting from MCA occlusion in Sprague–Dawley rats (Zhang and Iadecola, 1993a). Although SNP is widely used as a NO donor (Feelisch, 1991), this agent may have pharmacological actions that are not related to NO production. For example, SNP inhibits NMDA receptors by producing ferrocyanide ions (Kiedrowski et al., 1992). Furthermore, the vasodilator effect of SNP cannot be accounted for entirely by NO production (Feelisch, 1991). In the present study, we have demonstrated that SIN 1, a more specific NO donor (Feelisch, 1991; Manzoni et al., 1992), exerts a protective effect virtually identical to that of SNP. These observations therefore provide stronger evidence that NO is involved in the response. In addition, the results of the present study demonstrate that the protective effect of NO donors is well preserved in SHRs. Since SHRs are more refractory

than Sprague–Dawley rats to treatments aimed at reducing stroke size (e.g., Reis et al., 1991), the findings indicate that the protection afforded by NO donors is indeed substantial.

The effect of NO donors cannot be attributed to differences in the physiological variables of the rats because AP, blood gases, and body temperature were carefully controlled and did not differ among groups. The reduction in infarct size is unlikely to be due to brain hypothermia (Morikawa et al., 1992c) or to changes in plasma glucose (Prado et al., 1988) because (a) administration of NO donors does not influence brain temperature and (b) NO donors produce only a slight increase plasma glucose, a change that cannot account for the protective effect. However, because brain temperature was not monitored in experiments in which the MCA was
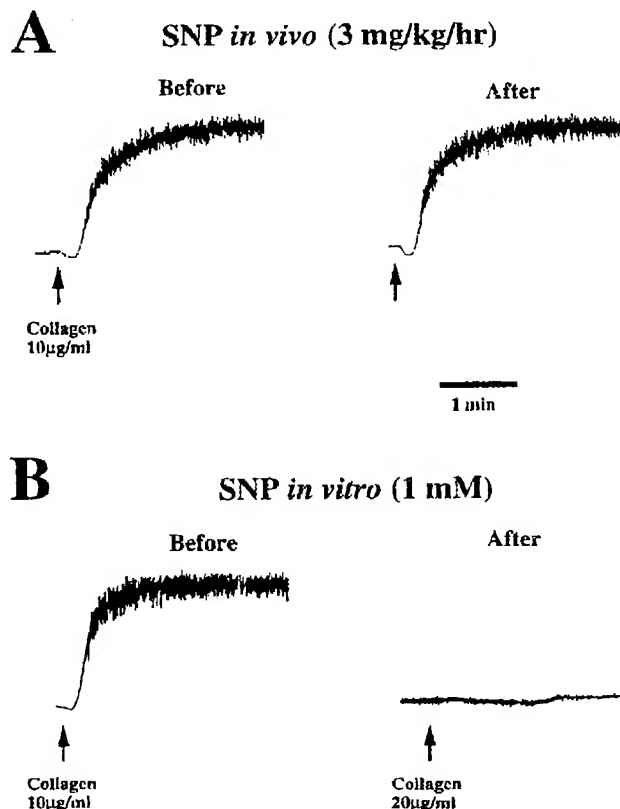

FIG. 6. Effect of sodium nitroprusside (SNP) on platelet aggregation using collagen as an aggregating agent. Platelet function was assessed ex vivo on platelet-rich plasma using an aggregometer. A: Platelet aggregation was studied before and after intracarotid administration of SNP (3 mg/kg/h for 1 h). SNP infusion did not inhibit platelet aggregation to collagen (10 μg/ml). B: In contrast, when SNP (1 mM) was added directly to platelet-rich plasma of untreated donor rats, platelet aggregation to collagen (20 μg/ml) was blocked. The results suggest that SNP at doses that are effective in attenuating the infarct resulting from middle cerebral artery occlusion does not influence collagen-induced platelet aggregation. However, SNP applied in large concentrations to platelet-rich plasma inhibits platelet aggregation.

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occluded, the participation of brain hypothermia cannot be completely ruled out. The reduction in infarct size cannot result from the infusion of phenylephrine needed to counteract the hypotension induced by NO donors, because similar amounts of phenylephrine were administered to rats that received papaverine, a treatment that was not effective. Similarly, the reduction in the size of the infarct cannot be due to hemodilution resulting from the infusion of the drug because the total volume of fluid administered was small and did not influence arterial hematocrit or resting CBF. Although ventilation with 100% O_2 and resulting hyperoxia could have shortened the biological half-life of NO (Moncada, 1992), the fact that NO donors produce vasodilation and reduce stroke size indicates that the NO availability is sufficient for this agent to exert its biological effects. It is therefore likely that the attenuation of ischemic damage exerted by SNP or SIN 1 is not an artifact of the experimental procedure, but is due to an effect of these agents on ischemic damage.

Several independent lines of evidence suggest that NO is beneficial in the initial stages of cerebral ischemia. First, administration of L-arginine, the precursor of NO (Moncada, 1992), increases CBF to the ischemic territory and attenuates focal cerebral ischemic damage (Morikawa et al., 1992a,b). Second, stimulation of the cerebellar fastigial nucleus, a procedure that increases neocortical CBF by local release of NO (Iadecola, 1992b), improves CBF in the ischemic tissue and attenuates the infarct after MCA occlusion (Zhang and Iadecola, 1993b). Third, transection of the parasympathetic cerebrovascular fibers arising from the sphenopalatine ganglia, a nerve system that may synthesize NO perivascularly (Toda and Okamura, 1990), enlarges the size of the infarct resulting from MCA occlusion (Kano et al., 1991). Fourth, some laboratories, but not all (cf. Nowicki et al., 1991; Buisson et al., 1992; Nagafuji et al., 1993), have reported that NO synthase inhibitors enlarge the size of the infarct (Yamamoto et al., 1992; Zhang and Iadecola, 1993a). Finally, the present study demonstrated that NO-producing agents reduce focal ischemic damage. Collectively, these observations indicate that NO, either produced endogenously or administered exogenously, attenuates focal cerebral ischemic damage.

An important question concerns the mechanisms by which NO ameliorates the tissue damage resulting from focal ischemia. NO is a potent cerebrovasodilator (see Iadecola, 1993, for review) and, as such, could enhance collateral flow in the ischemic territory. This hypothesis is supported by our find-

ing that NO donors increase CBF in the ischemic tissue that is rescued from infarction. Thus, at variance with papaverine, NO increases CBF to the ischemic territory and may prevent infarction of tissue that is marginally perfused. NO could also reduce focal ischemic damage by inhibiting platelet aggregation (Radomski et al., 1991). Inhibition of platelet function could prevent microvascular occlusion in regions of stasis and improve microvascular flow. However, we have demonstrated that SNP, at a dose that reduces stroke size, does not affect platelet aggregation. This observation suggests that SNP reduces stroke size without affecting platelet function. However, the possibility that NO inhibits platelet function in vivo at the microvascular level cannot be entirely ruled out on the basis of our experiments because we tested platelet aggregation ex vivo.

Another potential mechanism by which NO could ameliorate focal ischemic damage relates to the fact that NO inhibits NMDA receptors (Manzoni et al., 1992). This agent, like other NMDA receptor antagonists, could reduce focal ischemic damage by "protecting" the tissue from NMDA-mediated injury (Choi, 1990). However, the effects of NO on NMDA receptors and on NMDA-mediated neuronal death remain highly controversial (see next paragraph). Therefore, whether NO protects neurons from ischemic damage by "cytoprotective" rather than "vascular–hemodynamic" mechanisms remains to be established. Whatever the mechanisms of the protection, the brain tissue retrieved from infarction is located between the ischemic core and nonischemic tissue, a region corresponding to the anatomical location of the so-called ischemic penumbra (Astrup et al., 1981). Neurons in the penumbra are electrically silent but are still able to maintain ionic gradients and can regain function if perfusion is restored (Astrup et al., 1981). Thus, NO may ameliorate ischemic damage by rescuing the ischemic penumbra from infarction.

NO is also a toxic substance (Nathan and Hibbs, 1991; Dawson et al., 1992), and several studies have suggested that this agent participates in NMDA-mediated neurotoxicity and hypoxic–ischemic damage (Dawson et al., 1991; Nowicki et al., 1991; Buisson et al., 1992; Nagafuji et al., 1992). Thus, NO may play both a beneficial and a deleterious role in cerebral ischemic damage. This "double-edged" role of NO is similar to that proposed for this agent in other organ systems (Schmidt et al., 1992; see also Beckman, 1991). The mechanisms of the opposing effects of NO in cerebral ischemic damage and their pathobiological significance are unclear at the present time. One hypothesis is the following:

In the early phases after induction of ischemia, NO could produce vasodilation, enhance collateral flow to the penumbra, and limit the extent of tissue damage. After several hours of ischemia, astrocytes become reactive and activated macrophages and leukocytes begin to accumulate in the ischemic territory (Garcia et al., 1993). These cells are endowed with an inducible isoform of NO synthase that, at variance with the constitutive neuronal and endothelial enzymes, is calcium independent and synthesizes NO continuously (Nathan and Hibbs, 1991; Murphy et al., 1993). Large fluxes of NO produced by these cells may aggravate the metabolic state of the tissue and worsen ischemic damage. Therefore, in the early stages after focal ischemia, NO may have a beneficial effect, while at later times this agent may be detrimental to the viability of the tissue. This hypothesis, however, needs to be tested experimentally.

The finding that NO donors enhance blood flow to the ischemic territory and attenuate focal ischemic damage suggests that these agents could be used in the treatment of acute ischemic stroke. One potential problem with using these agents in stroke patients is that they produce hypotension. In patients with myocardial infarction, a condition in which NO donors appear to be beneficial, these hemodynamic effects have been counteracted successfully with vasoconstrictors (Nitz and Fiedler, 1987). A similar approach could be used in patients with acute cerebral ischemia. NO donors may therefore have a role in the management of cerebral ischemia in the "hyperacute" phase. These agents could be used in conjunction with other therapeutic interventions, such as thrombolysis (Marshall and Mohr, 1993), to limit the degree of ischemia.

In conclusion, we have demonstrated that administration of NO donors after MCA occlusion improves CBF in the ischemic territory and reduces the tissue damage. The tissue rescued from infarction corresponds to the region in which NO donors increase CBF, suggesting that these agents exert their protective action by enhancing CBF to the ischemic territory. This effect was shown to be independent of inhibition of platelet aggregation by NO. The findings indicate that NO donors are beneficial in the acute stages of focal cerebral ischemia and raise the possibility that these agents could have a role in the management of acute ischemic stroke.

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